Inducing Somatic Embryos of Soybean *Glycine max* and *Glycine soja* on variation of sukrosa concentrations.

Endah Wahyurini

Agrotechnology, Agriculture Faculty, Universitas Pembangunan Nasional "Veteran", Yogyakarta, 55283, Indonesia

ABSTRACT

Production and productivity of Soybean in Indonesia has not been national demand. The government program to improve produvtivity and Soybean production to achieve self sufficience on 2015. To achieved self sufficience Soybean the most amount, short time and the same genotipe is by tissue culture technique. Genotipe and sukrosa in the medium tissue culture is important inducing somatic embryos. The objectives of this experiment were to inducing somatic embryos of Glycine max of varieties Anjasmoro and Glycine soja of varieties Mallika at the variation sukrosa concentration for MS medium by in vitro. The research by using immature cotyledons explant which was conducted at Greenhouse and Biotechnology Laboratory, Agrotechnology of UPN "Veteran" Yogyakarta, Indonesia from Mei to October 2010. The experiment with two factors was arranged in factorial Completely Randomized Design and 10 replications. One factor is explants soybean were : *Mallika* (*Glycine soja*) and *Anjasmoro* (*Glicyne max*). Two factors is concentration sukrosa were : 20 g/l. 30 g/l, and 40 g/l. Data were subjected to an analysis of variance followed by Bunnet's Honest Different Test (BHDT) at 5% significance level. The results showed that material explant Glycine max and Glicyne soja not induced somatic embryos. The best sukrosa concentrations 20 g/l for MS medium increased to time of embryos (days), growth procentage of embryos and fresh weight callus. The combination treatment Glicyne max and sukrosa 20 g/l to increased dry weight callus.

Keyword : Embryos somatic, soybean, sukrosa

INTRODUCTION

The Soybean is a crop that has rich nutritional and includes 10 crop commodities besides rice and corn commodities. In recent years, soybean production is still the range of 600-700 thousand tons per year, while demand has reached 2.0 million tons. The low national production of soybean, as well as the total area of plantations is still limited or declining, as well as productivity per unit area remains low. This is caused by the use of low-quality seeds and by the onset of the disease (Anonymous, 2010).

Propagation in the conventional soybean plants generally require a long time as well as a vast place so it needs to be done in biotechnology is by vegetative propagation of plants through tissue culture techniques *(in vitro).* Technology is an *in vitro* culture techniques in

plant breeding pieces of tissue in a sterile artificial media. The technology is based on the properties of the cell that each individual is able to form a new whole that has properties identical to the parent cell, especially the young (Wahyurini, 2008). The medium used for cultivating the tissue sections containing foods such as macro elements and micro nutrients. In addition, in the medium was also added source of carbon derived from sucrose, vitamins and growth regulators that serve to spur growth and improve the ability of cells to multiply and develop into a candidate plant (Gamborg and Shyluk, 1981 and George and Sherington, 1984)

Regeneration of plant tissue culture can be done through somatic embryogenesis and organogenesis. Somatic embryogenesis is widely used because it can accelerate the discovery of the success of transgenic crops with a high opportunity for transformation of somatic embryos can be derived from a somatic cell. Somatic embryos can be induced directly from tissue explants or indirectly through a callus phase. Plant regeneration from callus cultures often show genetic diversity that somatic embryogenesis is more efficiently used directly in the application of biotechnology for plant breeders.

Currently somatic embryogenesis are well known as regeneration induction to way of tissue culture explants, or indirectly through a callus phase. Its success is largely determined by media formulations optimized for each stage of culture (Yusnita, 2003). The successful regeneration of soybean plants is also highly dependent on the genotype used. From previous research studies, induction of somatic embryogenesis in peanut mostly done by using several concentrations of sucrose still produce a diverse number of embryos. So, on that ground, conducted research on somatic embryos soybean of white and black, are still sefamily with peanuts as Leguminoceae. The research to know about the concentration of sucrose is right for the formation of soybean embryos. Problems in this study is on how the provision konsentrsi sucrose effect on increasing the amount of soybean embryos and in a short time of planting material a bit.

MATERIALS AND METHODS

This research has been conducted in the greenhouse and laboratory Agroteknologi Department of Biotechnology in May until October 2010. Materials used include: soybean seed varieties *Anjasmoro* and *Mallika*, polybags, sand, manure, MS medium (Murashige and Skoog), agar, sucrose, 2.4 D, desinfeltan (Furadan, agrimycin, Benlate, 96% alcohol, bayclin 50%, sublimat 0.1%), sterile distilled water, aluminum foil, filter paper, gloves, and

detergents. Tools used include: the culture bottles, beakers, petridis, pH sticks, Laminair Air Flow (LAF), disentect sets, lighting Bunsen and autoclave.

The experiment was conducted using a design Randomized complete with 2 factors and four replications. First factor is the explant material (genotype soybean) comprising 2 level : white soybean Anjasmoro (K1) and black soybean Mallika (K2). Whereas Second factor is the concentration of sucrose, which comprises three level: 20 g / l (S1), 30 g / l (S2) and 40 g / l (S3). The data were analyzed by Variance Analysis at the level 5%. To know there are real differences between the treatment then the test by *Bunnet's Honest Different Test (BHDT)* at 5% significance level.

RESULTS AND DISCUSSION.

The results of the present analysis shows that the treatment appears embryo explant material significantly affect the concentration of sucrose but the treatment did not significantly affect time of embryos. Average value the time of embryos can be seen in Table 1

Table 1. The mean time of embryos (days)

Treatment	S1 (20 g/l)	S2 (30 g/l)	S3 (40 g/l)	rerata
K1 (white soybean)	22,25	22,25	24,75	23,167 a
K2 (black soybean)	25,50	26,25	26,25	26,00 b
rerata	23,88 p	24,38 q	25,25 q	(-)

Note book: Mean followed by same small letter indicate no real difference in the test BHDT 5%. Sign (-) showed no interaction

Table 1 shows that treatment of explants real K1 faster as the time of embryo than K2 treatment. At treatment concentrations of sucrose showed a real S1 faster when compared treatments S2 and S3. In the early growth response of black soybean callus showed a faster growth than white soybean. In morphology the size and shape of white soy beans Anjasmoro greater than Mallika black soybeans, so cotiledon as food reserves could supply the cells forming the meristem cells.

The embryo appears at day 22 after planting some sucrose treatment. Provision of sucrose with a concentration of 20 g / l markedly more rapid time of the embryo, this is due to sucrose with 20 g / l is the best carbon source that acts as a raw material producing energy in the process of respiration (Katuuk, 1984). It is this energy that is used in cell-cell division to form embryos.The results of the analysis of the growth procentage of embryos showed that

the treatment material did not significantly, but the concentration of sucrose significantly. Average value of the the growth procentage of embryos can be seen in Table 2.

Table 2. The mean of the growth procentage of embryos

Treatment	S1 (20 g/l)	S2 (30 g/l)	S3 (40 g/l)	rerata
K1 (white soybean)	81,25	75,00	81,25	79,17 a
K2 (black soybean)	87,50	57,50	68,75	71,25 a
rerata	84,38 p	66,25 r	75,00 q	(-)

Note book: Mean followed by same small letter indicate no real difference in the test BHDT 5%. Sign (-) showed no interaction

Table 2 shows that the treatment was not significantly different explants K1 with K2 treatment. At treatment concentrations of sucrose showed a greater percentage of real S1 explants are capable of forming embryos than treatment S2, and S2 greater than S3 real. There is no interaction between sucrose concentration and explant material percentage of explants capable of forming embryos.

Treatment of different materials genotipnya explants showed no real difference in the percentage of embryos. Soybean embryo explants of black and white soybeans has the ability and equal opportunity for the proliferation or growth of cells so that the percentage of growing embryo no real difference. The key to success in the formation of callus tissue of life is the existence of a sterile nutrient medium that has an optimum and suitable environment and culture (Ursila, 2004).

Provision of sucrose with a concentration of 20 g / l the real percentage of the growth of embryos than other treatments. The success of plant tissue culture is highly dependent on the media used. Tissue culture media not only provide macro and micro nutrients but also the carbohydrates that in general the form of sugar. Sugar is a source of carbon instead of carbon usually obtained plants from the atmosphere in the form of CO2 into a component for photosynthesis (Gunawan, 1988).

The results of the analysis of the number of embryos per explant explants showed that the treatment material and the concentration of sucrose does not significantly affect the number of embryos per explant The average number of embryos per explant can be seen in Table 3.

Table 3. Average number of embryos per explant

Treatment	S1 (20 g/l)	S2 (30 g/l)	S3 (40 g/l)	rerata
K1 (white soybean)	31,39	29,67	32,21	31,09 a
K2 (black soybean)	27,29	27,75	37,34	30,79 a
rata-rata	29,34 p	28,71 p	34,78 p	(-)

Note book: Mean followed by same small letter indicate no real difference in the test BHDT 5%. Sign (-) showed no interaction

Table 3 shows that treatment of explants K1 and K2 are not significantly different. At the concentration of sucrose treatment S1, S2 and S3 are not significantly different between treatments. There is no interaction between sucrose concentration and explant material to the average number of embryos per explant.

Treatment of different materials genotipnya explants showed no real difference in the number of embryos per explant. In forming callus growth, white soy faster growth, but the subsequent development of both materials explants showed the same ability to form embryos. This is due to the development of cells forming embryonic cells is influenced by the nutrients contained in the media.

Provision of sucrose with a concentration of 20 g / l apparent greater number of embryos per explant compared to other treatments. This is because sucrose is an important carbon source used as a constituent of cells, cell division, cell enlargement and differentiation of cells that can form the plant shoots, and embryos as well (George and Sherrrington, 1984). The results of the analysis of wet weight of explant material menunjukakn that the treatment effect is not real, but the treatment concentration of sucrose significantly affect callus wet weight. Average value of wet weight of callus can be seen in Table 4.

Table 4 shows that treatment of explants K1 and K2 are not significantly different in the concentration of sucrose treatment showed S1, kalusnya markedly more severe than the S2 and S3. There is no interaction between sucrose concentration and explant material to wet weight of callus.

Table 4. The mean wet weight of callus (g)

Treatment	S1 (20 g/l)	S2 (30 g/l)	S3 (40 g/l)	rerata
K1 (white soybean)	1,365	1,142	1,212	1,240 a
K2 (black soybean)	1,281	1,098	1,177	1,186 a
rerata	1,323 p	1,120 q	1,195 q	(-)

Note book: Mean followed by same small letter indicate no real difference in the test BHDT 5%. Sign (-) showed no interaction

Treatment of different materials genotipnya explants showed no real difference in wet weight of callus. In forming callus growth, white soy faster growth, but subsequent developments showed the same ability proliferating. This is due to the development of callus formation and embryo are fixed, so the plant will produce the same wet weight (Andarwening, 2009).

On a wet weight parameters of callus that the granting of the concentration of sucrose 20 g / l showed the greatest callus wet weight compared with other treatments. The state thus induced cells in tissue explants grown on media with the addition of sucrose 20 g / l more rapidly receive the nutrients necessary for its development, while also influenced by the ability of the plant itself in receiving nutrients. Growth in the general sense is the formation, among others, the volume size, weight and number of cells (Salisbury and Ross, 1992). Dry weight analysis results showed that treatment of explant material and the concentration of sucrose does not significantly affect the dry weight of callus. There is interaction between the materials treated explants with sucrose concentration on dry weight of callus. Average value of dry weight of callus can be seen in Table 5.

Table 5. The mean dry weight of callus (g)

Treatment	S1 (20 g/l)	S2 (30 g/l)	S3 (40 g/l)	rerata
K1 (white soybean)	1,734 ab	1,722 b	1,769 a	1,742
K2 (black soybean)	1,699 b	1,530 c	1,701 b	1,643
rerata	1,717	1,626	1,735	(+)

Note book: Mean followed by same small letter indicate no real difference in the test BHDT 5%. Sign (-) showed no interaction

Table 5 shows that the combination treatment K1S3 (white soybean and sucrose 40 g / l) did not differ significantly with treatment K1S1 (white soybean and sucrose 20 g / l) and

significantly different from the other treatment combinations on the dry weight of callus. Combination treatment K2S2 (black soybeans and sucrose 30 g / l) were significantly lighter weight than other treatments. According Septiana (2010) that the process of explant growth and development can be realized by the accumulation of which would tranlokasion asimilat into various cell explants require. If explants are not able to form sufficiently asimilat explants were then growth will very.

K1S3 combined treatment (white soybean and sucrose 40 g / l) and K1S1 (white soybean and sucrose 20 g / l) produced the greatest dry weight of callus compared to other treatments. Dry weight of callus showed progression and cell growth. The white soybeans in sucrose concentration of 20 or 40 g / l produced the greatest dry weight of callus may be due to the density concentration and induces the cells to maintain the acidity of H + so that the water potential in the cell down and eventually enter the cell and place cell development. Provision of sucrose as a substitute for carbon is very involved in the process of photosynthesis, so the number asimilat formed in the formation of many organs of plants (George and Sherrrington, 1984).

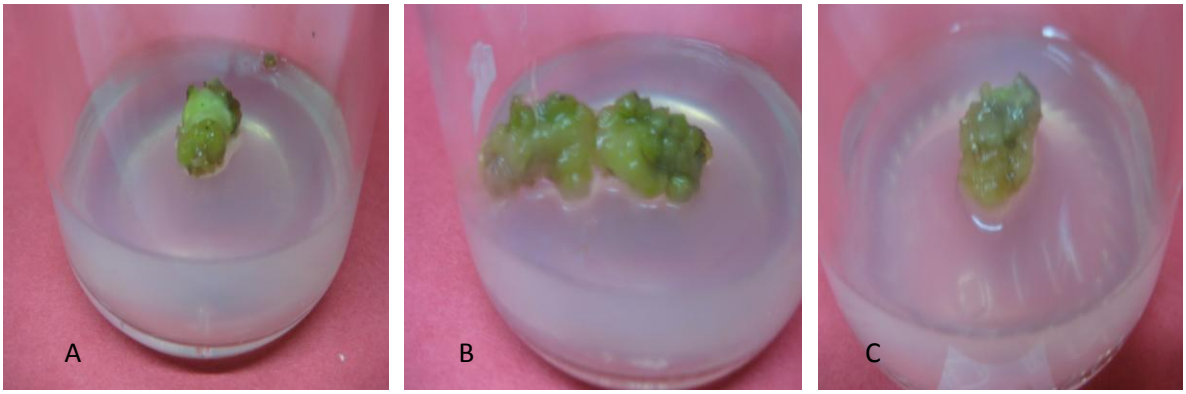

Figure 3. Embryo somatic with treatment K1S1 (A) after three weeks ; K2S1 (B) after four weeks ; K1S1 (C) after ten weeks

CONCLUSION

1. Treatment of white soybean (K1) and black soybeans (K2) does not affect the induction of somatic embryos *in vitro*.
2. The treatment concentration of sucrose 20 g / l may increase increased to time of embryos (days), growth procentage of embryos and fresh weight callus.

3. The combination treatment Glicyne max and sukrosa 20 g/l to increased dry weight callus.

ACKNOWLEDGEMENT

This project is under the financial aid of the Higher Education Research Grant Young Lecturer of DP2M in 2010, so the authors present gratitude here.

REFERENCES

Anonymous. 2010. Balitkabi. Assessment Development and Technological Innovation Toward Self-Sufficiency Soybean Current Year 2014. National Conference Soybeans: Malang June 29.

Andarwening, F. 2009. Induction of callus and shoots formation of Soybean hipokotil Anjasmoro, Arjasari and Manglayang in concentration 0.5 mg / L 2,4 D and several concentrations of BAP in In Vitro Cultures. UNPAD Bandung. Pp 32

Gamborg, O. L and J. P. Shyluk. 1981. Nutrition, Media. and Characteristic of Plant Cell and Tissue Culture. Thorpe, T.A. (Ed.). Plant Tissue Culture, Methods and Application in Agiculture. New York. Academic Press. Pp 44.

George, E. F. and P. D. Sherrington. 1984. Plant Propagation by Tissue Culture. Handbook and Directory of Commercial Laboratories. England.

Gunawan, L. W. 1988. Tissue Culture Techniques. Plant Tissue Culture Laboratory of Biotechnology ITB PAU Bogor. Department of General Education and Culture. Pp. 79.

Katuuk, J. R. P. 1984. Micropropagation Techniques in Plant Tissue Culture. Ministry of Education and Culture. Jakarta

Salisbury, F. B. and C. W. Ross. 1992. Plant Physiology. Warsds Worth Publishing Company. California. Hal: 74-270.

Septiana, E. 2010. Various responses Sucrose Concentration on Growth of Black Soybean Explants Crop (Glycine Soja) Varieties Mallika in vitro. UPN "Veteran" Yogyakarta. Unpublished Research Report.

Ursila, P. 2004. Effect of NAA concentration in MS media on callus growth of melon (Cucumis melo L.) Faculty of Agriculture, UPN "Veteran" Yogyakarta research report is published. Pp 45

Wahyurin, E. 2008. Effect of Sucrose in Sweet Corn Seed Improvement Potential Through embryo culture. Proceedings of the National Conference on Seed and Institutional Development Universitas National "Veteran" Yogyakarta: 10-11 November 2008. Pp. 18.

Yusnita. 2003. Tissue Culture: How to Reproduce Plants in Efficiently. Agromedia Persada: Jakarta.